



Allergic immune-regulatory effects of Adlay Bran on an OVA-immunized mice allergic model

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ARTICLE INFO

Article history:

Received 28 September 2011

Accepted 6 July 2012

Available online 15 July 2012

Keywords:

Allergy

Flavonoids

Adlay bran

Th2 cytokines

Anti-OVA IgE

ABSTRACT

Allergy is an inflammation associated with an elevated T helper (Th) 2 lymphocyte responses to allergens and elevated serum IgE levels and cytokines. In one of our previous studies using a cell model, various flavonoids were found to be involved the anti-inflammatory effects of adlay bran. The present study investigated the effect of the ethyl-acetate fraction of ethanolic extract of adlay bran (ABE-EtOAc) in an ovalbumin (OVA)-immunized murine model. Six-week-old female BALB/c mice underwent OVA sensitization and were used as an allergy model. An orogastric gavage was used to force feed these mice with 240 mg/kg ABE-EtOAc from their sixth week through their twelfth week. Immune reactions were determined by measuring changes in Th2-type cytokine (IL-4 and IL-5) levels and production of antibodies. ABE-EtOAc was found capable of regulating the Th1/Th2 immune reaction through its regulation of IL-2 and IL-4. It also significantly reduced the production of anti-OVA IgE antibodies (10%), increased the secretion of IFN- γ and decreased the secretion of IL-6 (38%). These results suggest that adlay bran extract can reduce an allergic reaction by balancing Th1/Th2 immune responses and that it might be used in the treatment of this condition.

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1. Introduction

Allergy, an immune dysfunction, has become increasingly prevalent in the developed countries (Ono, 2000). The existence of Th1/Th2 subsets in T-helper lymphocytes that differ in their cytokine secretion patterns and effector functions can provide a framework for understanding normal and pathological immune responses (Stern et al., 2005). CD4⁺ effector T cells, which produce Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) mediate the development of allergic immune responses (Romagnani, 2004). IL-4 is the major inducer of class-switching of Ig to B lymphocyte IgE biosynthesis associated with allergic responses (Poulsen and Hummelshøj, 2007). Because corticosteroids, the most commonly used drugs

treat allergies, have some side effects (Popescu, 2003), it might be of some use to find a dietary regimen to regulate the immune system, reduce the symptoms of allergies, and avoid the waste of drugs (Cross et al., 2001; Li et al., 2004; Ouweland, 2007; Takano et al., 2004).

Adlay (Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf), a common food in Asia, has been reported to have various anti-inflammatory (Chen et al., 2011; Seo et al., 2000) and anti-allergic (Chen et al., 2010, 2012; Hidaka et al., 1992; Hsu et al., 2003) effects. In one of our previous studies, we found that 20% dehulled adlay diet could suppress the production of IgE against ovalbumin (OVA) antigen and modulate the Th1/Th2 immune responses by reducing the secretion of IL-5 and increasing the production of IL-2 (Hsu et al., 2003). Furthermore, we have found adlay bran to be rich in flavonoids (Chen et al., 2011). The adlay bran extract suppressed the histamine and cytokine release in rat basophils shows their anti-allergic activity *in vitro* (Chen et al., 2012).

In this study, we further investigated the anti-allergy effects of adlay bran by dividing adlay seeds into hull, testa, and dehulled adlay, and further divided the edible dehulled adlay into bran and endosperm. We measured changes in Th2 cytokine and antibodies, including against ovalbumin (OVA) antigens, in different groups of mice with induced allergies: some untreated, some treated with other substances, and others treated different doses of adlay extract.

Abbreviations: ABE, the ethanolic extracts of adlay bran; ABE-EtOAc, the ethyl-acetate fraction from ethanolic extracts of adlay bran; CMC, carboxymethylcellulose; CE, catchin equivalents; ConA, concanavalin A; Ig, immunoglobulin; IFN- γ , interferon-gamma; IL, interleukin; OVA, ovalbumin; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; TNF- α , tumor necrosis factor-alpha.

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2. Materials and methods

2.1. Materials

Carboxymethylcellulose (CMC), concanavalin A (Con A), ovalbumin (OVA), phosphate-buffered saline (PBS), phytohemagglutinin (PHA) and others chemicals were obtained from Sigma Chemical (St. Louis, USA). Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) was obtained from Biological Industries (Haemek, Israel).

2.2. Preparation of the ethyl-acetate fraction of adlay bran ethanolic extract

The ethyl-acetate fraction of adlay bran ethanolic extract was obtained following previously reported procedures (Chen et al., 2011). Briefly, adlay bran powder (2 kg) was extracted three times with 20 L of ethanol at room temperature for 2 weeks. The dry extract (ABE, 365 g) was suspended in 4 L of H₂O, followed by several partition processes conducted using various extraction solvents: *n*-hexane, ethyl acetate, and 1-butanol. The resulting fractions were ABE-Hex (*n*-hexane soluble fraction), ABE-EtOAc (ethyl-acetate soluble fraction), ABE-BuOH (1-butanol soluble fraction), and ABE-H₂O (water soluble fraction), respectively. The ABE-EtOAc was concentrated under reduced pressure in a rotary vacuum evaporator to remove the solvent and obtain 145 g of dry extract (yield = 39.7%). The ABE-EtOAc was then dissolved in PBS containing 0.5% CMC and filtered through a Millipore microfilter (0.22 µm) for sterilization before oral administration. The components of ABE-EtOAc have been characterized by HPLC/MS (Supplementary Data, Fig. S1).

2.3. Experimental animals and feeds

Inbred female BALB/c mice (6 weeks old) were purchased from the BioLasso (Ilan, Taiwan). They were housed individually and fed a standard laboratory diet (Lab Rodent Chow Die 5001, Ralston Purina Co.) *ad libitum*. The mice were weighed weekly and food consumption measured daily. Animals were cared for and handled following the guidelines set forth by the Animal Care Committee of National Taiwan University (permission number: NTU-97-EL-56) and NIH Guidelines for the Care and Use of Laboratory Animals. Forty-eight mice were randomly divided into five groups: (1) PBS Control (*n* = 4), sham immunized and PBS p.o., (2) OVA Control (*n* = 11), immunized and PBS p.o., (3) Quercetin (*n* = 11), immunized and administered 10 mg/kg/day quercetin p.o., (4) 1X ABE-EtOAc (*n* = 11), immunized and administered 240 mg/kg/day ABE-EtOAc p.o., and (5) 2X ABE-EtOAc (*n* = 11), immunized and administered 480 mg/kg/day ABE-EtOAc p.o. All mice but the PBS controls were immunized three times with OVA in alum adjuvant every 2 weeks according to a previous study with some modification (Wegmann and Hauber, 2010). Briefly, mice were first immunized by intraperitoneal injection of 10 µg OVA (Grad V; Sigma, MO, USA)/200 µL in PBS with 20 mg aluminum hydroxide (Sigma-Aldrich, MO, USA) as the adjuvant. Two weeks later, they received a booster injection (20 µg OVA plus 30 mg alum adjuvant). Two weeks later, they received another such booster injection. After booster injection for 2 weeks, blood was collected from the retro-orbital venous plexus and centrifuged at 12,000g for 20 min. Serum was collected and stored at −80 °C for future assays. A schematic diagram of the treatment schedule is shown in Fig. 1.

2.4. Measurement of serum total and OVA-specific antibodies level

Total and OVA-specific antibodies were measured using a commercial ELISA kit (Bethyl, TX, USA) following manufacturer's directions. Briefly, 96-well plates were coated with 10 µg/mL of anti-mouse Ig or OVA solution in 0.1 M NaHCO₃ buffer (pH 8.2). After overnight incubation at 4 °C, the plates were washed three times with PBS solution and blocked with BSA-PBS [PBS containing 1% BSA] for 1 h at room temperature. They were then washed three times with PBST [PBS containing 0.05% (v/v) Tween 20]. 100 µL of test sera diluted with BSA-PBS buffer was added to the wells and incubated for 2 h at room temperature. Plates were then washed with PBST three times, and HRP-conjugated anti-mouse Ig diluted in BSA-PBS buffer was added. The plates were then incubated at room temperature for 2 h. At the end of the 2 hr incubation period, the plates were washed three times with PBST and added with 100 µL of detection antibodies and further incubated for 1 h. After the hour-long incubation, the plates were washed five times with PBST and each well was added with 100 µL of TMB (3,3',5,5'-tetramethylbenzidine). The plates were left in the dark for 30 min at room temperature. Finally, 100 µL of 1 M H₂SO₄ was added

to each well and mixed well. Absorbencies were measured at 405 nm with an OPTImax Tunable Microplate Reader (Molecular Devices, Sunnyvale, USA). For total antibodies level, the results were calculated using a standard curve. For OVA-specific antibodies levels, the results were expressed in ELISA units (EU): $EU = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{positive}} - A_{\text{blank}})$.

2.5. Measurement of phagocytosis and NK cell activity

The phagocytosis activity of monocytes was evaluated using the Phagotest Kit (Opregen Pharma, Heidelberg, Germany) according to manufacturer's directions and measured by flow cytometry (Filias et al., 2011). The NK cell activity was evaluated using the NK Cell Activity Kit (Asia Bioscience, Taipei, Taiwan) following manufacturer's directions (Piriou et al., 2000).

2.6. Preparation of splenocytes

After sacrificed, the splenocytes were collected from the spleen of mice was carried out according to the method described (Lin et al., 2006). Briefly, the spleen was collected from the mice, and grinded to get the single cell suspension. After depletion of red blood cells by treated with RBC lysing buffer, the splenocytes were suspended in DMEM medium supplemented with 10% FBS and the antibiotic-antimycotic for subsequent experiments.

2.7. Measurement of cytokine levels in splenocytes

The concentrations of IL-2, IFN-γ, IL-4 and IL-5 in splenocytes were measured using a commercial ELISA kit (eBioscience, San Diego, USA). Briefly, splenocytes (5×10^6 cells/well) from immunized mice were isolated and suspended in 10% FBS/DMEM culture medium. They were then cultured with ConA (5 µg/mL), PHA (20 µg/mL) or OVA (25 µg/mL) for 48 h at 37 °C in a CO₂ incubator. After incubation, supernatants (100 µL) were transferred to a plate with 96 wells pre-coated the antibodies (anti-IL-2, anti-IFN-γ, anti-IL-4 and anti-IL-5 capture antibody) and incubated at room temperature. After 2 h incubation, plates were washed three times with PBST and added with HRP-conjugated detection antibodies (100 µL/well) for 2 h at room temperature. After incubation, 100 µL of TMB was added to each well and the plate left in the dark for 30 min at room temperature. Finally, 100 µL of 1 M H₂SO₄ were added to each well and mixed well. Absorbencies were measured at 405 nm with an OPTImax Tunable Microplate Reader (Molecular Devices, Sunnyvale, USA).

2.8. Preparation of peritoneal macrophages

Before sacrificed, murine peritoneal macrophages were collected from the peritoneal cavity of mice according to previous procedure (Chen et al., 2011). Briefly, 10 mL of ice-cold DMEM was injected into mice by an intraperitoneal (i.p.), and then collected these DMEM as peritoneal fluid. After washing with RBC lysis buffer, peritoneal macrophages were centrifuged and grown in DMEM supplemented with 10% FBS and the antibiotic-antimycotic. Cells were seeded at 5×10^6 cells/well and allowed to adhere for 2 h at 37 °C in 5% CO₂. After non-adherent cells were removed by washing with PBS, adherent cells were used for subsequent experiments.

2.9. Measurement of inflammatory cytokine levels in peritoneal macrophages

The concentrations of IL-6 and TNF-α in peritoneal macrophages were measured as previously described (Chen et al., 2011). Briefly, peritoneal macrophages were challenged with LPS (1 µg/mL) or OVA (25 µg/mL) for 48 h. Then, concentrations of inflammatory cytokines in the supernatants were measured using a commercial ELISA kit (eBioscience, San Diego, USA).

2.10. Statistical analysis

Results are expressed as the mean ± standard deviation (SD) for three experiments. Differences between specific means were analyzed by one-way analysis of variance (ANOVA) using the SPSS system, version 11.0 (SPSS, Chicago, IL, USA). Group means were compared using one-way ANOVA followed by Duncan's multiple range test. *p*-values less than 0.05 were considered significant.

3. Results

3.1. Effects of ABE-EtOAc on humoral immune responses

To evaluate humoral immune response, we measured total serum Ig antibody levels. As shown in Table 1, OVA-immunized mice were found to have significant higher IgE levels than the controls (1045 ng/mL vs. 149 ng/mL), while both low dose and high dose ABE-EtOAc-treated group had lower IgE level than the OVA-immunized groups (761 ng/mL vs. 1045 ng/mL and 682 ng/mL vs.

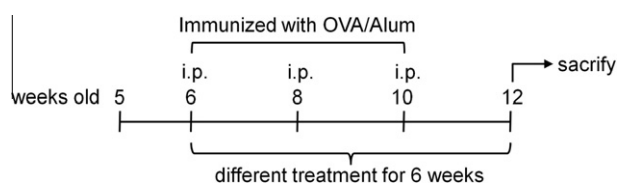


Fig. 1. OVA-immunized mice model and treatment with ABE-EtOAc.

Table 1

The effects of different treatments on the serum total antibodies of OVA-immunized BALB/c mice.

Group	IgA	IgM	IgG	IgG ₁	IgG _{2a}	IgE
			mg/mL			ng/mL
PBS control	0.59 ± 0.06	1.22 ± 0.15a	0.99 ± 0.15a	0.13 ± 0.03a	0.15 ± 0.03ab	149 ± 027.2a
OVA control	0.61 ± 0.06	1.52 ± 0.32b	2.21 ± 0.38b	1.66 ± 0.26c	0.10 ± 0.02a	1045 ± 296.5d
Quercetin	0.65 ± 0.10	1.41 ± 0.25ab	2.11 ± 0.41b	0.99 ± 0.18b	0.15 ± 0.03ab	973 ± 259.0cd
1X ABE-EtOAc	0.65 ± 0.07	1.22 ± 0.28a	2.20 ± 0.36b	1.43 ± 0.69bc	0.19 ± 0.03b	761 ± 165.3bc
2X ABE-EtOAc	0.61 ± 0.08	1.23 ± 0.27ab	2.81 ± 0.71c	1.55 ± 0.59c	0.31 ± 0.11b	682 ± 191.4b

^aEach value represents mean ± S.D.^{*}Values followed by the different letters are significantly different by Duncan's multiple range test ($p < 0.05$).

1045 ng/mL, respectively, both $p < 0.05$). The administration of 240 mg/kg ABE-EtOAc significantly reduced total IgE by twenty-seven percent, while 480 mg/kg was found to bring about a further reduce it by 35%.

To evaluate the effects of ABE-EtOAc on Th1 and Th2 responses, we measured the IgG subclass antibodies IgG₁ and IgG_{2a}. ABE-EtOAc reduced IgG₁ levels (14%) and increased IgG_{2a} level (90%), suggesting that the oral supplementation of adlay bran extract downregulated the IgE level in serum and possibly modulated the Th1/2 balance though its effect on modulating IgG₁ and IgG_{2a} levels.

3.2. Effects of ABE-EtOAc on innate immune responses

Innate immune responses defend the body against foreign antigens, including bacterial antigens. To ensure that the anti-allergic effects of ABE-EtOAc would not suppress healthy immune responses, we investigated the effect of ABE-EtOAc on the phagocytosis and NK cell activity. Neither low dose (240 mg/kg) nor high dose (480 mg/kg) of ABE-EtOAc affected the phagocytosis (Table 2). Nor was it found to reduce NK cell activity. Together, these findings suggest that adlay bran extract does not influence the general immune function.

3.3. Effects of ABE-EtOAc on Allergy Immune Responses

We evaluated the effect of ABE-EtOAc on allergy immune responses. To do this we compared its effect on serum levels of OVA-specific IgE and IgG₁ in OVA-immunized mice with those of PBS-immunized mice (Fig. 2). The OVA-immunized mice were found to have substantial increases in these two antibodies. Of course, it is natural for the immune system to produce an allergic response upon initial exposure to an antigen. After 6 weeks' oral supplementation, compared to the untreated OVA-immunized mice, those treated with ABE-EtOAc had significantly reduced OVA-specific IgE and IgG₁ levels, reduce by 10% and 12%, respectively. Moreover, it increased the OVA-specific IgG_{2a} levels by 161%. To study its affect on a positive control, we administered

Table 2

The effects of different treatments on the phagocytosis and the NK cell activity of OVA immunized BALB/c mice.

Group	Phagocyte activity (%)	NK activity (%)	
		E/T = 20/1	E/T = 40/1
PBS control	24.6 ± 3.0	20.6 ± 1.7	23.3 ± 2.5a
OVA control	20.5 ± 5.5	22.3 ± 2.4	27.2 ± 3.8ab
Quercetin	24.2 ± 7.5	23.9 ± 2.3	28.2 ± 2.9b
1X ABE-EtOAc	24.5 ± 5.0	22.2 ± 3.3	26.0 ± 3.5ab
2X ABE-EtOAc	24.4 ± 6.4	22.6 ± 2.5	27.4 ± 3.4ab

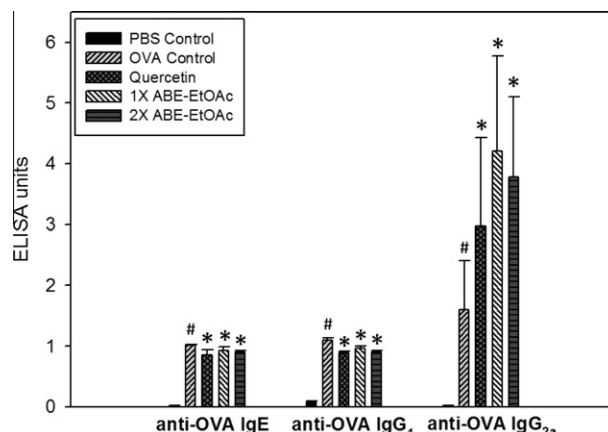
^aEach value represents mean ± S.D.^{*}Values followed by the different letters are significantly different by Duncan's multiple range test ($p < 0.05$).

Fig. 2. The effects of different doses of ABE-EtOAc on the serum OVA-specific antibodies in OVA-immunized BALB/c mice. The PBS Control group received PBS only. The OVA control group were OVA-immunized mice. The Quercetin group were OVA-immunized mice treated with quercetin (10 mg/kg, p.o.). The 1X ABE-EtOAc group were OVA-immunized mice treated with ABE-EtOAc (240 mg/kg, p.o.). The 2X ABE-EtOAc group were OVA-immunized mice treated with ABE-EtOAc (480 mg/kg, p.o.). #Significant difference from PBS Control, $p < 0.05$ and *significant difference from OVA control, $p < 0.05$.

quercetin, a phytochemical rich in food, to the mice. Oral supplementation of quercetin reduced the OVA-specific IgE and IgG₁ levels and increased the IgG_{2a} levels (16%, 19%, and 86%, respectively), a finding consistent with those of a previous study (Park et al., 2009).

We wanted to explore the possible mechanism underlying adlay bran extract's effect on allergy. To do this, we measured Th1 cytokines (IL-2 and IFN- γ) and Th2 cytokines (IL-4 and IL-5) in splenocytes. The splenocytes in OVA-immunized mice were not found to have much change in Th1 cytokine secretion, while those in mice fed the high dose of ABE-EtOAc (480 mg/kg) had significant increases in the IL-2 levels (40%), though no such change was found in those of the mice fed the low dose (Fig. 3a). Furthermore, ABE-EtOAc tended to increase IFN- γ levels ($0.05 < p < 0.1$) (Fig. 3b). The OVA-immunized mice had marked increases in IL-4 and IL-5 levels (Fig. 3c and d). Mice fed ABE-EtOAc (240 mg/kg or 480 mg/kg) had significantly reduced Th2 cytokine levels, particularly IL-4, which was reduced by 30 percent. However, only the administration of quercetin (10 mg/kg) was found to significantly reduce IL-5 levels. This finding suggests adlay bran extract can suppress the specific Th2 immune responses, not all.

3.4. Effects of ABE-EtOAc on inflammatory immune responses

To evaluate the anti-inflammatory effects of ABE-EtOAc, we measured IL-6 and TNF- α level in peritoneal macrophages. OVA-immunized mice had significantly increased levels of IL-6, indicating that inflammation had developed (Fig. 4a). In peritoneal macrophages cultured with either OVA (25 μ g/mL) or LPS

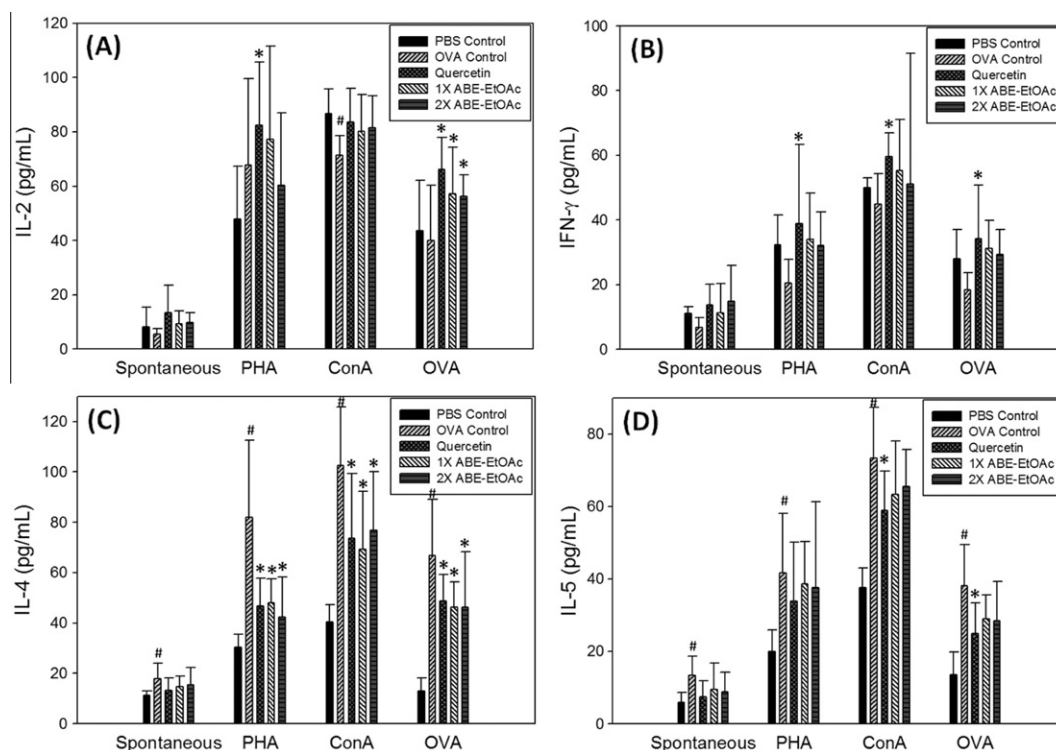


Fig. 3. The effects of different doses of ABE-EtOAc on the cytokine secretion of splenocytes obtained from OVA-immunized BALB/c mice. The splenocytes (5×10^6 cells/well) were cultured in a medium with the absence or presence of PHA (20 μ g/mL), ConA (5 μ g/mL) or OVA (25 μ g/mL) for 48 h. The detection limits for IL-2, IFN- γ , IL-4, and IL-5 were 2, 4, 4, and 15 pg/mL, respectively. #Significant difference from PBS Control, $p < 0.05$ and *significant difference from OVA control, $p < 0.05$.

(1 μ g/mL), IL-6 levels had increased 2-fold and 10-fold, respectively. In ABE-EtOAc mice, IL-6 levels were reduced to half of those of the supplement groups. It is interesting to note that the peritoneal macrophages of ABE-EtOAc treated groups had increased levels of TNF- α levels (Fig. 4b), and when the peritoneal macrophages of the high dose ABE-EtOAc group were cultured with LPS, TNF- α was inhibited by 25%. Together, these findings suggest that adlay bran extract suppresses the over-expression inflammatory cytokines.

4. Discussion

The results of this study show that adlay bran extract can be used safely with some animals, and has an anti-allergic and anti-inflammatory effect *in vivo*, can mediate the allergy *via* modulate Th1/Th2 immune responses.

Oral supplementation with adlay bran extract appeared to be healthy in these mice, which showed no pathological signs or abnormalities during the feeding period. We found no significant differences in their body weight gain, feed intake, or the rate of growth (Supplemental Data, Table S1). Nor were liver function, AST, or ALT affected (Supplemental Data, Table S2). It had no effect on phagocytosis and NK cell activity, suggesting that it is probably not harmful to normal defense mechanisms against pathogenic infection (Table 2). There were no signs of toxicity in the mice fed high dose of adlay bran extract (480 mg/kg).

Allergen-specific IgE mediates a type I hypersensitivity, triggering inflammatory responses in local tissue and eventually generating various allergic symptoms (Owen, 2007). Th2 cells, a subset of CD4⁺ T cells, have been found to play an important role in the OVA-immunized mice model by their influence on IL-4 and IL-5 production (Hamid and Minshall, 2000). IL-4-induced switching of Ig to IgE and IgG₁ are well-known allergic responses (Secrist et al.,

1993). In the current study, IL-4 levels were increased by 5-fold in splenocytes obtained from OVA-immunized mice, but by feeding them ABE-EtOAc (240 mg/kg), the IL-4 levels could be significantly decreased (Fig. 3c). ABE-EtOAc also significantly reduced serum IgE and IgG₁ levels, indicating that adlay bran extract can suppress Th2 immune responses. The simultaneous increase in IgG_{2a} level showed that adlay bran extract also enhanced Th1 immune responses (Fig. 2). Compared with IL-4, the productions of IL-5 were not effect by adlay bran extract. Although the genes for IL-4 and IL-5 are closely located on chromosome, there is evidence for the unique transcriptional regulation of IL-5 that is independent of AP-1 binding (Mori et al., 1999). Moreover, the c-Maf pathway only controls IL-4 but not IL-5 expression in stimulated stage (Dong et al., 2001). Based on this information, it can be thoroughly explored the mechanism of adlay bran on the Th2 cell. However, the critical role of IL-4 in the development and maintenance of the Th2 phenotype has been highlighted recently. Moreover, the mast cells play a central role in the immediate type of allergic response through the release of mediators *via* degranulation. We had been reported that the adlay bran extracts reduced the release of histamine and inhibits the IL-4 secretion *via* suppress the phosphorylation of Akt (Chen et al., 2012). These results may suggest that ABE-EtOAc also inhibits the activation of mast cells and subsequent histamine release.

Allergic inflammation is caused by inflammatory cytokines such as IL-6 and TNF- α (Alam, 1997). In a previous study, we found adlay bran extract capable of inhibiting inflammation in the LPS-induced peritoneal macrophages (Chen et al., 2011). When trying to assess the anti-inflammatory effect *in vivo*, in this study, we found that adlay bran extract reduced the production of cytokines which have been associated with allergic responses. For example, the over-expression of IL-6 has been reported to increase allergic responses by overcoming CD4⁺CD25⁺ Treg suppression (Doganci et al., 2005), and TNF- α , an inflammatory cytokine, is reported to be released during allergic responses as well (Chai et al., 2011).

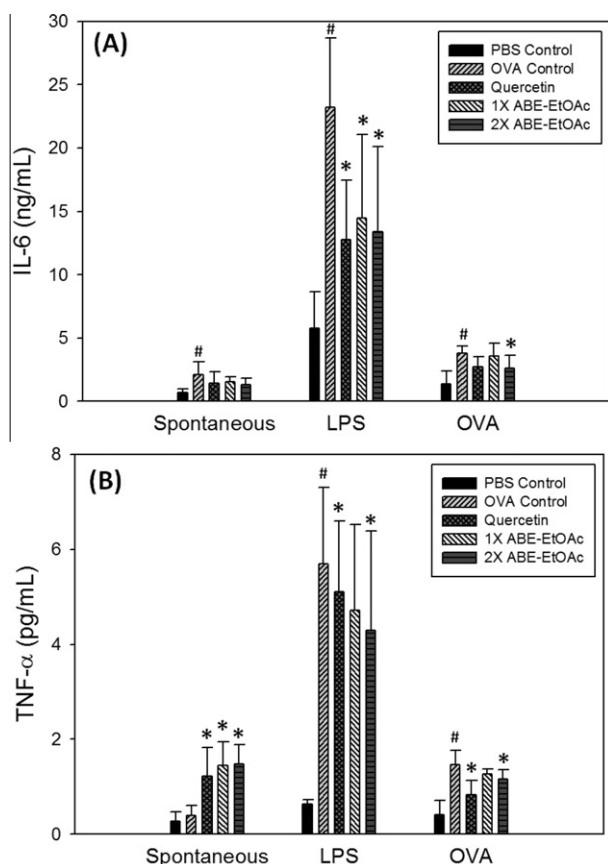


Fig. 4. The effects of different doses of ABE-EtOAc on the cytokine secretion of peritoneal macrophages obtained from OVA-immunized BALB/c mice. The macrophages (5×10^6 cells/well) were cultured in a medium with the absence or presence of LPS ($1 \mu\text{g/mL}$) or OVA ($25 \mu\text{g/mL}$) for 48 h. The detection limits for IL-6 and TNF- α were 4 and 8 pg/mL, respectively. #Significant difference from PBS Control, $p < 0.05$ and *significant difference from OVA control, $p < 0.05$.

Previous murine studies investigating the role of TNF- α in allergy have demonstrated an important role for TNF- α in contributing to allergen induced inflammation, and Th2 cytokine production (Nakae et al., 2007). TNF- α influences allergic responses by increasing mast cell and eosinophil activity and inducing inflammation via NF- κ B pathway. In this study, the animal fed oral supplements of ABE-EtOAc produced less IL-6 and TNF- α , suggesting that the supplementation reduced inflammation (Fig. 4). However, the production of TNF- α were increased in ABE-EtOAc treated groups without any stimulation. The inflammatory transcription factor, NF- κ B, can be activated by TLR4, TNFR or IL-1R (Verstrepen et al., 2008). This may explain that ABE-EtOAc can specific suppress the TLR/MyD88/TRAF6 pathway to regulated the production of TNF- α . But more experiments need to clarify the effects of adlay bran on the TNF/TNFR pathway. Taken together, oral supplementation with adlay bran extract suppressed not only T cell-mediated immune responses but also inflammatory responses *in vivo*. In other words, adlay bran's modulation of immune responses alleviated allergic responses in our model.

There are a few studies reporting that some cereals have anti-allergic effects. (Iguchi et al., 2007) have demonstrated that fermented barley extract possesses can exert an anti-allergic effect by suppressing the differentiation in Th2 cells. Lee et al. (Lee et al., 2006) reported that ethanolic extract of black rice could suppress airway inflammation associated with asthma. These studies were not using the same active components as we were in this study of the effect of adlay extract on allergy. We found in one of our previous studies that dehulled adlay could suppress allergic

reactions by modulating Th1/Th2 responses (Hsu et al., 2003). In this study, we separated bran from endosperm and measured phenol and flavonoid content in the bran. These components were characterized by LC/MS which show the involvement of various flavonoids in ABE-EtOAc (Chen et al., 2011). Like quercetin, flavonoids exhibit a strong anti-allergic and anti-inflammatory effect (Gonzalez-Gallego et al., 2007; Kawai et al., 2007). We studied the effect of feeding ABE-EtOAc flavonoid-enriched fraction ($\sim 74.5 \text{ mg CE/g}$ of extracts) to mice. ABE-EtOAc significantly suppressed IgE levels in serum and IL-4 levels in splenocytes, exhibiting an anti-allergic effect. Therefore, the bran of adlay, a flavonoid-rich cereal holds some potential a food to help treat allergies.

In conclusion, ABE-EtOAc supplementation led to significant suppression of serum OVA-specific IgE and IgG₁ levels in sera and suppression of secretion of IL-4 secreted from the splenocytes of OVA-immunized mice, while not affecting the normal immune function. Overall, these results suggest that oral administration of adlay bran extract can ameliorate allergic responses without side effects. The adlay bran extract can potentially be used as a functional food aiding the treatment of allergic responses.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fct.2012.07.011>.

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